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0012155919 BIOSIS NO.: 199900415579
Metabolic characteristics of a human hepatoma cell line stably transfected with hormone-sensitive lipase
AUTHOR: Pease Richard J (Reprint); Wiggins David; Saggerson E David; Tree Jeni; Gibbons Geoffrey F
AUTHOR ADDRESS: Department of Biochemistry and Molecular Biology, University College London, Gower Street, London, WC1E 6BT, UK**UK
JOURNAL: Biochemical Journal 341 (2): p453-460 July 15, 1999 1999
MEDIUM: print
ISSN: 0264-6021

DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Clones of *HepG2* cells were selected that stably express the cDNA for hormone-sensitive lipase (*HSL*). When cells were cultured in the presence of labelled extracellular oleate, accumulation of labelled fatty acid as cellular triacylglycerol (TAG) was significantly lower in the...

...excess TAG fatty acid releasedin the transfected cells underwent intracellular re-esterification to TAG prior to oxidation. The results suggest that fatty acids mobilized by *HSL* are directed immediately into the oxidative pathway and are not available for biosynthetic processes. It appears likely, therefore, that intracellular TAG-derived fatty acids which...
DESCRIPTORS:
ORGANISMS: *HepG2* cell line (Hominidae...

2/3,K/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0011253251 BIOSIS NO.: 199800047498
Species-specific alternative splicing generates a catalytically inactive form of human hormone-sensitive lipase
AUTHOR: Laurell Henrik; Grober Jacques; Vindis Cecile; Lacombe Thierry; Dauzats Michele; Holm Cecilia; Langin Dominique (Reprint)
AUTHOR ADDRESS: Unite INSERM 317, Inst. Louis Bugnard, Fac. Med., Univ. Paul Sabatier, Hop. Rangueil, F-31403 Toulouse Cedex 4, France**France
JOURNAL: Biochemical Journal 328 (1): p137-143 Nov. 15, 1997 1997
MEDIUM: print
ISSN: 0264-6021
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Hormone-sensitive lipase (*HSL*) catalyses the rate-limiting step of adipose tissue lipolysis. The enzyme is also expressed in steroidogenic tissues, mammary gland, muscle tissues and macrophages. A novel *HSL* mRNA termed hHSL-S, 228 bp shorter than the full-length *HSL* mRNA, was detected in human adipocytes. hHSL-S mRNA results from the in-frame skipping of exon 6, which encodes the serine residue of the...
...cells showed neither lipase nor esterase activity but was phosphorylated by cAMP-dependent protein kinase. hHSL-S mRNA was found in all human tissues expressing *HSL*, except brown adipose tissue from newborns. It represented approx. 20% of total *HSL* transcripts in human subcutaneous adipocytes. No alternative splicing was detected in other mammals. Human and mouse three-exon *HSL* minigenes transfected into primate and rodent cell lines reproduced the splicing pattern of the endogenous *HSL* genes. Analysis of hybrid human/mouse minigenes transfected into human cell lines showed that cis-acting elements responsible for the skipping of human exon 6...
DESCRIPTORS:
...ORGANISMS: *HepG2* (Hominidae

2/3,K/3 (Item 1 from file: 149)
DIALOG(R)File 149:TGG Health&Wellness DB(SM)

02058418 SUPPLIER NUMBER: 83139534 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Transcriptional regulation of adipocyte hormone-sensitive lipase by glucose.
Smih, Fatima; Rouet, Philippe; Lucas, Stephanie; Mairal, Aline; Sengenes, Coralie; Lafontan, Max; Vaulont, Sophie; Casado, Marta; Langin, Dominique

Diabetes, 51, 2, 293(8)
Feb,
2002
PUBLICATION FORMAT: Magazine/Journal; Refereed ISSN: 0012-1797
LANGUAGE: English RECORD TYPE: Fulltext TARGET AUDIENCE: Professional
WORD COUNT: 6461 LINE COUNT: 00527

TEXT:

Hormone-sensitive lipase (*HSL*) catalyzes the rate-limiting step in the mobilization of fatty acids from adipose tissue, thus determining the supply of energy substrates in the body. *HSL* mRNA was positively regulated by glucose in human adipocytes. Pools of stably transfected 3T3-F442A adipocytes were generated with human adipocyte *HSL* promoter fragments from -2,400/ +38 to -31/+38 bp linked to the luciferase gene. A glucose-responsive region was mapped within the proximal promoter...
...two GC-boxes in the -137-bp region. Cotransfection of the -137/+38 construct with USF1 and USF2 expression vectors produced enhanced luciferase activity. Moreover, *HSL* mRNA levels were decreased in USF1- and USF2-deficient mice. Site-directed mutagenesis of the *HSL* promoter showed that the GC-boxes, although contributing to basal promoter activity, were dispensable for glucose responsiveness. Mutation of the E-box led to decreased...

Hormone-sensitive lipase (*HSL*) is a key enzyme for the hydrolysis of adipose tissue triacylglycerol into fatty acids that are the major source of body energy in the absence of dietary lipids. Catecholamines and insulin are important regulators of lipolysis in adipocytes through a modulation of intracellular cAMP levels and reversible phosphorylation of *HSL* (1). The lipase is thought to catalyze the rate-limiting step in cAMP-dependent lipolysis. In agreement, a strong linear correlation was found between *HSL* protein levels and the maximum lipolytic capacity of human subcutaneous adipocytes stimulated by a (beta)-adrenergic agonist (2). Moreover, targeted disruption of the *HSL* gene in the mouse results in blunted (beta)-adrenergic agonist-induced lipolysis (3,4). Clinical studies also support a role for *HSL* as a limiting factor in adipose tissue lipolysis and show that, besides the short-term modulation of activity by phosphorylation, variations in *HSL* expression are associated with changes in lipolytic capacity. Indeed, obese patients and normal-weight subjects with a family trait for obesity show decreased maximal lipolytic effect of catecholamines and blunted *HSL* expression (5,6). Furthermore, genetic studies suggest that *HSL* participates in the polygenic background of obesity and type 2 diabetes (7,8).

Increased mobilization of fatty acids from adipose tissue stores in diabetic patients...
...complication of type 1 diabetes (9). Several mechanisms may account for the enhanced lipolysis. Hypoinsulinemia and an increase in the concentrations of stimulatory hormones stimulate *HSL* activity via posttranslational modifications. Moreover, isolated fat cells from streptozotocin-induced diabetic rats show increased maximal lipolytic response to agents acting at the postreceptor level (10) and increased *HSL* mRNA and protein levels (11). Insulin deficiency and hyperglycemia could both account for the upregulation of *HSL* expression in diabetes. Insulin per se had no effect on *HSL* mRNA concentrations in 3T3-F442A murine adipocytes (12). However, in this cellular model, glucose deprivation resulted in a twofold decrease in *HSL* mRNA and total activity levels (13). The effect of glucose was reversible and was not due to an impairment of the differentiation program, since the...

...exposure of isolated rat adipocytes to glucose in the presence of insulin resulted in an increase of basal and stimulated lipolysis and a maintenance of *HSL* protein levels (14).

The adipocyte form of *HSL* is encoded by nine exons spanning 11 kb (15,16). The transcriptional start site has been mapped in a short 5'-noncoding exon located 1...

...region linked to the luciferase gene into rat adipocytes showed the existence of an active promoter (17). However, the regulatory elements within the human adipocyte *HSL* promoter have not been characterized.

Thus, we decided in this study to characterize the cis-acting regions that are critical for promoter activity and mediate the responsiveness to glucose.

RESEARCH DESIGN AND METHODS

Plasmids. Constructs containing human adipocyte *HSL* 5' flanking regions from -2,400 to -31 relative to the transcription start point and 38 bp of the 5' untranslated region in pGL3basic vector encoding a modified firefly luciferase (Promega, Madison, WI) have been described previously (17). Mutant *HSL* promoter constructs were generated by synthetic nucleotide assembly in pGL3basic using the KpnI site of the vector and the XbaI site of the *HSL* promoter. These constructs were made using standard procedures (18) and sequenced using a Perkin Elmer Dye terminator sequencing kit and an ABI 373 sequencer. Assembly...previously described (20). (beta)-Galactosidase expression was used to normalize transfection efficiency and was quantified using an o-nitrophenyl-(beta)-D-galactopyranoside colorimetric assay (18). *HepG2* and COS7 cells were cultured in DMEM containing 10% FCS, 200 units/ml penicillin, and 50 mg/l streptomycin. The cells were transfected with Fugene...

...Adipose primary cell culture total RNA was extracted using the Qiagen RNeasy kit. Total RNA was stored at -80 (degrees) C until analysis. Quantitation of *HSL* and cyclophilin mRNA levels were performed by reverse transcription-competitive PCR as described previously (21). The reverse transcription step was performed on 100 ng of...

...competitor. PCR products were separated by capillary electrophoresis and quantified using the ABI PRISM 310 Genetic Analyzer system with the Genescan program (PE Applied Biosystem). *HSL* mRNA levels were normalized with cyclophilin mRNA levels.

Preparation of nuclear extracts and electromobility shift assay. 3T3-F442A preadipocyte and adipocyte nuclear extracts were prepared...

...was fixed in 10% acetic acid/10% ethanol and dried. Gels were exposed and analyzed using a Molecular Dynamics SI445 Phosphorimager.

Northern blot analysis of *HSL* mRNA in USF1- and USF2-deficient mice.

Total RNA was isolated from perigonadal adipose tissue of fasted USF1- and USF2-deficient mice (23,24). Wild-type littermates were used as controls. Northern blot analysis was performed using mouse *HSL* (12) and ribosomal 18S cDNA probes as described previously (25). Specific signals were quantitated using a Phosphorimager (Molecular Dynamics).

Statistical analysis. The nonparametric Mann-Whitney U test for unpaired values was used to compare *HSL* mRNA levels. ANOVA with least-square difference post hoc test was used for comparisons of luciferase activities (SPSS). $P < 0.05$ was the threshold of significance.

RESULTS

Glucose-mediated regulation of *HSL* mRNA in primary cultures of human adipocytes. To establish that human *HSL* was regulated by glucose, a primary culture system was used to obtain human differentiated adipocytes. Cells were treated for 48 h in a serum-free medium containing 25 or 1 mmol/l glucose. *HSL* mRNA levels were quantified using reverse transcription-competitive PCR. The low glucose concentration led to a decrease in the ratio of *HSL* to cyclophilin mRNA levels from 0.049 (+ or -) 0.003 to 0.024 (+ or -) 0.004 ($P < 0.05$, $n = 3$).

Effect of extracellular glucose concentration on human *HSL* promoter activity in murine 3T3-F442A adipocytes. Because there is no established human preadipocyte cell line available, we used the mouse 3T3F442A preadipocyte cell line to analyze human *HSL* promoter activity in response to glucose. Luciferase constructs containing 2,400 to 31 bp of the 5' flanking region and 38 bp of the 5...observed at 15 mmol/l of glucose. The addition of FCS and insulin in the culture medium containing 25 mmol/l glucose did not modify *HSL* promoter activity.

(FIGURE 1 OMITTED)

EMSA of the potential binding sites in the -137-bp region. To determine more precisely the sequences involved in the...

...been shown to mediate glucose responsiveness (27).

(FIGURE 2 OMITTED)

EMSA was performed with the set of oligonucleotides described in Fig. 2. EMSA of the *HSL* E-box performed with adipocyte nuclear extracts showed two bands (Fig. 3). Competition with unlabeled oligonuceotide showed that the DNA-protein interaction was specific for...

...glucose-responsive genes harbor two E-boxes separated by 5 bp (29). Because a half E-box CACTGA is located 5 bp 3' from the *HSL* E-box (Fig. 2), we speculated that the sequence might be weakly bound by USF factors. However, no specific binding was observed using preadipocyte and...

...nuclear extracts or cellular extracts prepared from COS7 cells transfected with USF1 and USF2 expression vectors (data not shown).

(FIGURE 3 OMITTED)

EMSA of the *HSL* 5' GC-box (Fig. 4A) showed two bands that displayed a binding pattern characteristic of members of the Sp family of nuclear factors. The binding to the probe was specific, since competition was observed with the *HSL* 5' GC-box and the Sp1 control oligonucleotides but not with the unrelated HNF1 oligonucleotide. Antibody against Sp1 induced a weak supershift of the low...

...was able to totally supershift binding proteins in the high-mobility complex. Combination of the two antibodies led to the disappearance of the two bands. *HSL* 5' GC-box oligonucleotide did not completely abolish Sp1 binding to the probe, but competition with the Sp1 control oligonucleotide led to a complete disappearance of the bands. This indicated that Sp1 and Sp3 had a lower affinity for the *HSL* 5' GC-box than for the Sp1 control competitor. This observation is in accordance with previously published results showing a better affinity of Sp1 for GGGCGGG than for GGGTGGG motifs (30). The use of the probe corresponding to the *HSL* 3' GC-box with the ideal consensus sequence GGGCGGG led to the same pattern (Fig. 4B). Cross-competitions confirmed that *HSL* 3' GC-box was a better competitor of Sp1 and Sp3 binding than *HSL* 5' GC-box.

(FIGURE 4 OMITTED)

E- and GC-box mutagenesis analysis by transient transfection into adipocytes. To evaluate the functional importance of each of the boxes on *HSL* promoter activity in adipocytes, we performed single and multiple site-directed mutageneses of the sites. Lack of binding of the cognate factors to the mutated...

...shown). Mutant constructs were transiently transfected into primary rat adipocytes maintained in serum-free DMEM containing 25 mmol/l glucose (Fig. 5). Mutation of the *HSL* E-box induced a 50% decrease in luciferase activity. Mutation of the *HSL* 5' GC-box had a strong negative effect, whereas mutation of the *HSL* 3' GC-box had no effect. Combined mutation of the two GC-boxes led to lower luciferase activity than single mutations. Finally, mutations of the three boxes totally abolished luciferase activity.

(FIGURE 5 OMITTED)

Transactivation of the *HSL* promoter by transient cotransfection of expression vectors for USF1, USF2, and ADD1/SREBP1c. Because of the importance of the *HSL* E-box in promoter activity and its in vitro binding to USF, we wished to determine the transactivation properties of USF1 and USF2 on the *HSL* promoter. Cotransfection of the -137/+38 construct with USF1 and USF2 expression vectors in *HepG2* cells resulted in a marked increase in luciferase activity (Fig. 6). Similar results were obtained in COS7 cells (data not shown). USF1 and USF2 overexpression 6 OMITTED)

HSL gene expression in adipose tissue of USF1- and USF2-deficient mice. To further assess the role of USF1 and USF2 in adipocyte *HSL* gene expression, *HSL* mRNA levels were determined by Northern blot analysis of adipose tissue from USF1- and USF2-deficient mice (23,24). The amount of *HSL* mRNA was reduced to 49% in USF1-deficient mice and to 14% in USF2-deficient mice compared with wild-type mice (Fig. 7).

(FIGURE 7 OMITTED)

Glucose response analysis of *HSL* promoter mutants stably transfected into 3T3-F442A adipocytes. To define the DNA elements involved in the glucose response, the mutant constructs were stably transfected into ...

...did not alter the glucose response (data not shown). Therefore, the glucose response element could be attributed to the E-box in the human adipocyte *HSL* promoter.

(FIGURE 8 OMITTED)

Characterization of glucose metabolites involved in the modulation of *HSL* promoter activity. To determine the metabolic pathway(s) involved in the glucose response, we tested several glucose analogs and metabolites (Fig. 9). Compounds were added...

...expression has been reported for a growing set of genes involved in metabolic and energetic pathways in the cell (35). We have recently shown that *HSL*, the gene encoding the enzyme catalyzing the breakdown of triacylglycerol into fatty acids in adipose tissue, is positively regulated by glucose in a mouse adipocyte cell line (13). Here, we show that *HSL* mRNA is also responsive to glucose in primary culture of human subcutaneous adipocytes. Because the molecular mechanisms underlying the glucose response are largely unknown in the adipocytes, we sought to determine the cis-acting elements in the human adipocyte *HSL* promoter mediating upregulation by glucose.

Determination of reporter gene activity in differentiated 3T3-F442A adipocytes showed that the proximal 137 bp of the *HSL* promoter contained a glucose-responsive region. Interestingly, this glucose-responsive region contains two functional GC-boxes binding the Sp family of nuclear factors and an...

...the regulation of the leptin gene (39). However, our data do not support a role for Sp1 or Sp3 in the glucose response of the *HSL* promoter. Indeed, individual and double mutations of the GC-boxes did not alter the glucose response of several pools of stably transfected 3T3-F442A adipocyte ...

...nuclear extracts prepared from either glucose-deprived or glucose-fed adipocytes (data not shown). However, we showed that the GC-boxes are necessary for full *HSL* promoter activity in adipocytes.

A typical E-box motif, CACGTG, was located between -106 and -101 in the *HSL* promoter. USF1/USF2 heterodimers from adipocyte nuclear extracts bound to the E-box. Moreover, overexpression of the transcription factors increased the -137/+38 construct-mediated luciferase activity, and mutation of the E-box lowered promoter activity in rat adipocytes. The importance of USF was also observed in vivo. Indeed, *HSL* gene expression was decreased in adipose tissue of USF1- and USF2-deficient mice compared with wild-type mice. Together, these data demonstrate that USF1 and USF2 could participate in the transactivation of the *HSL* gene in adipose tissue. Mutation of the *HSL* E-box abolished the glucose response in stably transfected 3T3-F442A adipocytes. E-boxes have been identified as parts of glucose response elements for several...

...induction of hepatic genes. However, as previously reported (26), these transcription factors cannot by themselves explain the transcriptional regulation by glucose. USF binding to the *HSL* E-box did not differ between nuclear extracts from adipose cells grown in the presence or absence of glucose (data not shown). Our data show that the *HSL* E-box is an essential part of the glucose response element. Whereas USF may be indirectly involved in the transcriptional activation by glucose, the data ...induction of fatty acid synthase and acetyl CoA carboxylase gene expression (41). The lack of effect of 3-O-methyl glucose and 2-deoxyglucose on *HSL* promoter activity suggests that a metabolite downstream to glucose-6-phosphate in the glycolytic pathway was involved. However, dihydroxyacetone, which has been shown to increase hepatic glucagon receptor mRNA expression (42), did not restore *HSL* promoter activity. It has been proposed that glucose acts in liver though the nonoxidative branch of the pentose phosphate pathway to induce the L-pyruvate kinase gene expression (43). Xylitol, which is converted into xylulose 5-phosphate, was unsuccessful in restoring *HSL* promoter activity. The hexosamine pathway may also mediate the effect of glucose on gene expression, as proposed for leptin expression in skeletal muscle and

adipose tissue (39). The lack of glucosamine effect excluded this pathway and its end product UDP-N-acetylglucosamine in *HSL* transcriptional activation. Together, the data suggest that a metabolite generated during glycolysis between glucose-6-phosphate and triose phosphate could regulate the transcription of the human *HSL* gene. The fructose and mannose stimulatory effects are in agreement with this scenario, since they enter glycolysis at the glucose-6-phosphate level. Hence, the metabolic steps involved in the regulation of *HSL* promoter activity define a novel mechanism for the transcriptional effect of glucose.

In conclusion, we have shown that an E-box and two GC-boxes are critical cis-acting elements of the human adipocyte *HSL* promoter. USF, through binding to the E-box, may participate in the promoter transactivation. The E-box also mediates the glucose-mediated induction of *HSL* gene expression. Metabolism of glucose at a step between glucose-6-phosphate and triose phosphates is required. Further work is needed to identify the metabolites...June 2001 and accepted in revised form 5 November 2001.

DMEM, Dulbecco's modified Eagle's medium; EMSA, electromobility shift assay; FCS, fetal calf serum; *HSL*, hormone-sensitive lipase; USF, upstream stimulatory factor.

2/3,K/4 (Item 2 from file: 149)

02040798 SUPPLIER NUMBER: 80392831 (USE FORMAT 7 OR 9 FOR FULL TEXT)

The HIV protease inhibitor nelfinavir induces insulin resistance and increases basal lipolysis in 3T3-L1 adipocytes.(Statistical Data Included)

Rudich, Assaf; Vanounou, Sharon; Riesenberg, Klaris; Porat, Michal; Tirosh, Amir; Harman-Boehm, Ilana; Greenberg, Andrew S.; Schlaeffer, Francisc; Bashan, Nava

Diabetes, 50, 6, 1425(7)
June,
2001
DOCUMENT TYPE: Statistical Data Included PUBLICATION FORMAT: Magazine/Journal; Refereed ISSN: 0012-1797 LANGUAGE: English
RECORD TYPE: Fulltext TARGET AUDIENCE: Professional
WORD COUNT: 5370 LINE COUNT: 00457

... metabolism caused by HPIs at the cellular level. Using different agents and incubation periods, protease inhibitors have been shown to impair insulin signaling events in *HepG2* cells (18), to inhibit adipocyte differentiation (19, 20), and to decrease adipocyte insulin-stimulated glucose transport without affecting the insulin signaling cascade (21). Yet, despite...anti-phosphotyrosine (4G10) antibodies (purchased from Upstate Biotechnology (Lake Placid, NY)); anti-Ser 473 PKB antibody (from Cell Signaling (Beverly, MA)); and antihormone-sensitive lipase (*HSL*) and anti-perilipin antibody recognizing both the A and B isoforms (prepared as previously described) (22).

Immunofluorescent detection of GLUT4 in plasma membrane lawns. Cells...OMITTED)

Potential cellular mechanisms for nelfinavir-stimulated lipolysis and insulin resistance. Long-term regulation of lipolysis may involve alterations both in the expression level of *HSL* and in perilipins. The latter are believed to regulate lipolytic activity by limiting the access of *HSL* to the lipid droplets (28-30). Figure 3A demonstrates the effect of nelfinavir on the content of these two proteins. As shown in the upper blot, *HSL* protein content was not elevated in nelfinavir-treated cells compared with control cells, and it even tended to decrease with concentrations of 30 (micro)mol/l. This finding suggests that the increased basal lipolysis shown above (Fig. 2A) cannot be attributed to increased *HSL* expression. The lower blot demonstrates the protein content of the two isoforms of perilipin, A and B (molecular weight 57 and 46 kDa, respectively). A...differentiation program (20). In the present study, adipocyte differentiation was not directly assessed. Yet, GLUT4 protein

content was unaffected by nelfinavir treatment (Fig. 1B), whereas *HSL* content at the higher range of nelfinavir used, and perilipin also at lower concentrations, were found to be reduced (Fig. 3A).

Perilipins, located under basal conditions at the phospholipid interphase of the triglyceride droplet, are believed to limit *HSL* access to its substrate (28-30). Consequently, a reduction in their abundance results in higher *HSL* action on triglyceride storage pools. A recent perilipin-knockout model also largely supports an inhibitory role of perilipin on *HSL* action (23). Interestingly, in 3T3-L1 adipocytes, reduced perilipin content was previously suggested as a mechanism for the activation of lipolysis in response to tumor...67, 2000

(18.) Schutt M, Meier M, Meyer M, Klein J, Aries SP, Klein HH: The HIV-1 protease inhibitor indinavir impairs insulin-signalling in *HepG2* hepatoma cells. Diabetologia 43:1145-1148, 2000

(19.) Zhang B, MacNaul K, Szalkowski D, Li Z, Berger J, Moller DE: Inhibition of adipocyte differentiation by...

...deoxyglucose; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; FFA, free fatty acid; HAART, highly active antiretroviral therapy; HPI, HIV protease inhibitor; *HSL*, hormone-sensitive lipase; IRS, insulin receptor substrate; KRPB, Krebs-Ringer phosphate buffer; PBS, phosphate-buffered saline; PKB, protein kinase B;

2/3,K/5 (Item 3 from file: 149)

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Depot-specific differences in adipose tissue gene expression in lean and obese subjects.

Lefebvre, Anne-Marie; Laville, Martine; Vega, Nathalie; Riou, Jean Paul; Gaal, Luc van; Auwerx, Johan; Vidal, Hubert

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... lean and obese humans. We specifically investigated the expression of the genes that code for 1) the lipoprotein lipase (LPL) and the hormone sensitive lipase (*HSL*), two key enzymes controlling important aspects of fatty acid metabolism in adipocytes; 2) the insulin-sensitive GLUT 4, glycogen synthase (GS), and 6-phosphofructo-1...

...We have developed a multispecific competitor DNA molecule that allowed us to quantify precisely the levels of the mRNAs encoding GLUT4, GS, PFK-1, LPL, *HSL*, insulin receptor (total mRNA and mRNA variant with exon 11), IRS-1, PI-3K, and Rad(17). In addition to these mRNAs, we also set...of these, mRNAs. Significant differences were found for the mRNAs encoding GLUT4, GS, insulin receptor, leptin and PPAR(Gamma).

The mRNA levels of LPL and *HSL*, two essential enzymes of fatty acid metabolism in adipose tissue, were not different in omental and in subcutaneous depots. Among all the mRNA analyzed, these two RNAs were the most abundantly expressed in adipose tissues (LPL: 373 (+ or -) 61 vs. 368 (+ or -) 491 *HSL*: 468 (+ or -) 100 vs. 465 (+ or -) 83 amol/(Mu)g total RNA, in omental vs. subcutaneous fat). Furthermore, PI-3K (228 (+ or -) 39 vs. 267...not related to differences in the expression levels of the main enzymes of triacylglycerol hydrolysis. Indeed, the mRNA levels of LPL, and more importantly, of *HSL*, the key enzyme in intracellular lipolysis, were similar in omental and subcutaneous adipose tissue, both in lean and in obese subjects. Therefore, the increased lipolytic...1984

(23.) Kosaki A, Pillay TS, Xu L, Webster NJG: The B isoform of the insulin receptor signal more efficiently than the A isoform in *HepG2* cells. J Biol Chem 270:20816-20823, 1995

(24.) Okabayashi Y, Maddux BA, McDonald AR, Logson CD, William JA,

Goldfine ID: Mechanism of insulin-induced...
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